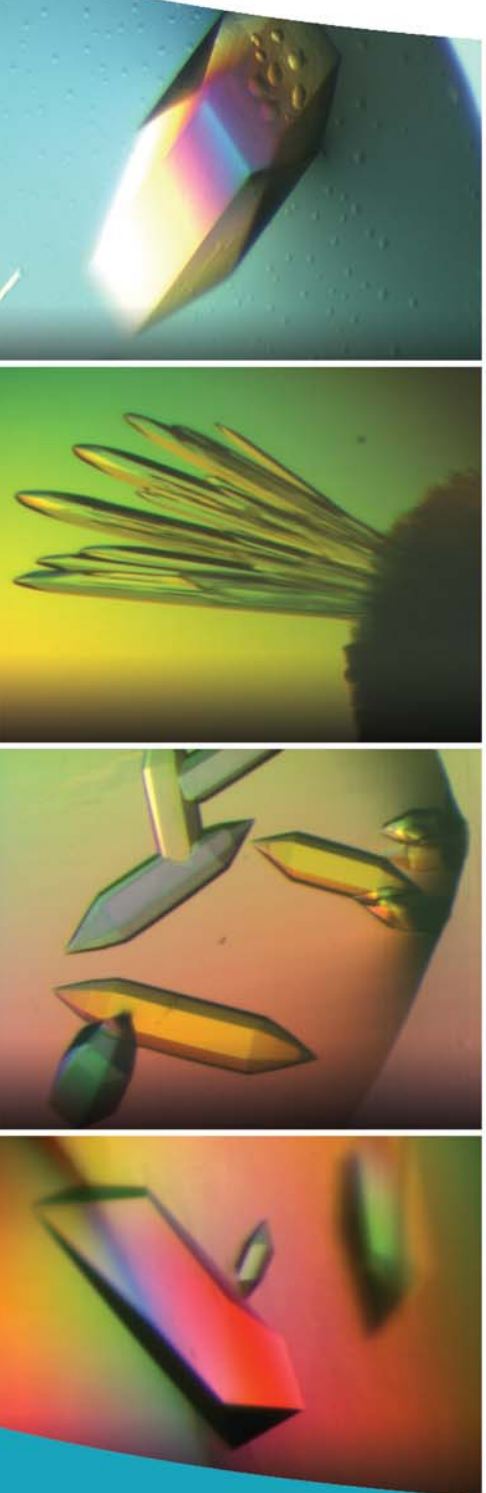
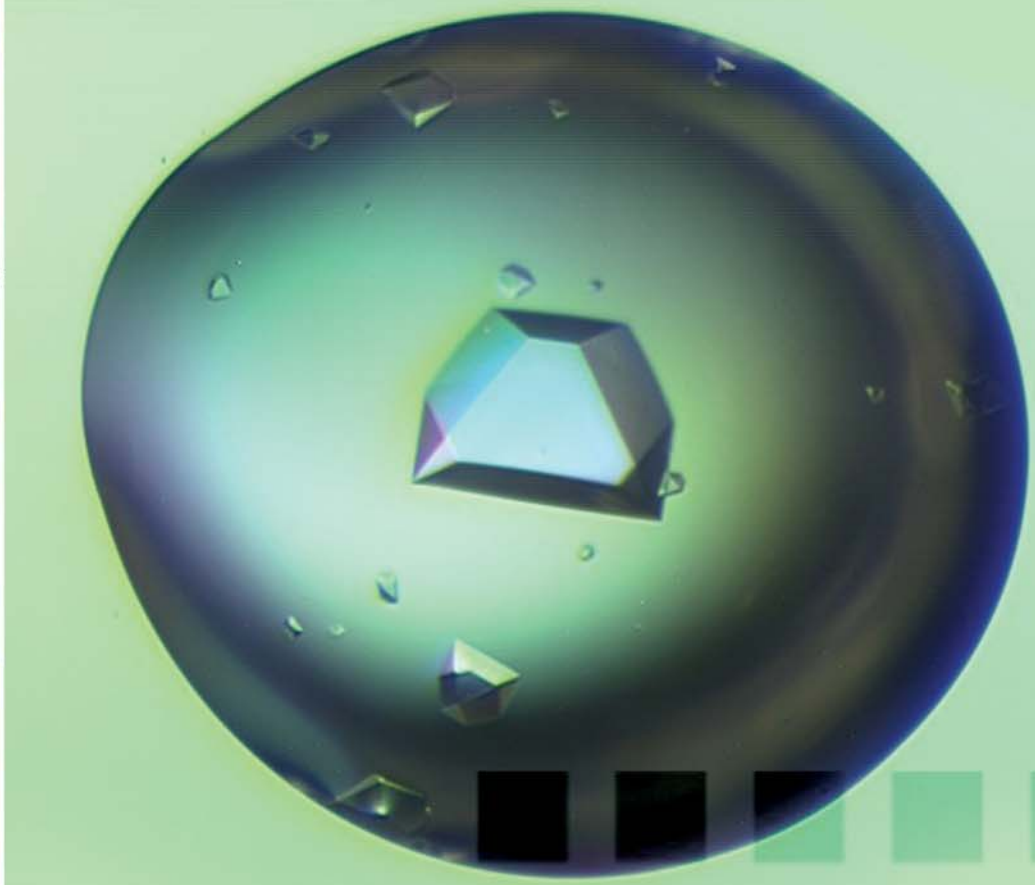
TTP LABTECH

# LabCrystal

MAKING PROTEIN CRYSTALLOGRAPHY SET-UPS EASIER





Welcome to TTP LabTech's protein crystallography newsletter. In this issue we talk to respected crystallographers from industry and academia around the globe to see how they have implemented automated protein crystallography set-up. We also look at how mosquito® has helped to improve accuracy, repeatability and reduce costs associated with crystallisation.

# why mosquito®?

mosquito® makes protein crystallography screening faster, more cost-effective and quite simply easier than ever before

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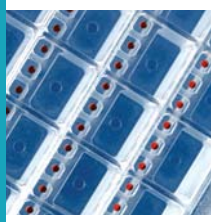
## Flexibility

Compatible with all standard crystallography plates, **mosquito's** disposable, positive displacement micropipettes can handle a wide range of viscosities.



**mosquito®** is the only instrument capable of automating both high density 96-well hanging drop and sitting drop plate preparation, as well as microbatch screens. Because it pipettes in columns, **mosquito®** can mirror the reservoir solution onto a plate seal, whilst its accurate small drop-size is ideal for both soluble and membrane proteins.

## Precision

**mosquito's** precise drop placement means drops are placed centrally in the sub-wells of sitting-drop plates, minimising issues with drops being distorted or not coinciding. This also facilitates easier identification of crystals for automated analysis.

## Repeatability

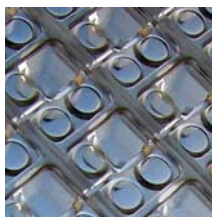
**mosquito's** accuracy and repeatability allows users to create several multi-component drops per well, even in 96-well hanging drop setups. These drops allow different constructs, volume ratios or protein concentrations to be assessed at the same time.

**mosquito®** is also capable of multiple aspirations before a single dispense. This technique is essential to automating additive screening and allows a combination of solutions to be dispensed simultaneously – with additional mixing if required – resulting in perfect drop formation with no protein evaporation. Multi-aspiration also enables precise dispensing of lower than normal volumes (down to 25 nl).

## Practical innovation

TTP LabTech's long experience of developing and commercialising robust technologies has put us in the forefront of drug discovery research. We work closely with scientists to understand the issues experienced on a daily basis. As a result, we have applied our expertise to assist in the development of products or consumables to overcome these problems. Some examples of these collaborations are show below.

## Nanolitre optimisation and screening made easy!



The new Gerresheimer Wilden **3 well crystallisation plate** is designed to SBS standard in a 96-well format and offers a combination of features not previously available in one plate:

- **Triple well** – for multiple conditions around a single reservoir. Triplicates allow for 288 constructs per plate
- **Easy crystal viewing and retrieval** – made from optically superior polymer (UVP) with a new well design for easy viewing under a microscope. Micro-numbering within wells allows simple orientation.
- **Wide volume range** – drop volume range is 10nl-5µl with reservoirs of 30-65µl
- **Better sealing** – wide, flat partitions between wells give a good seal, ensuring integrity of each well section

## More drops, more screens

TTP LabTech has developed a new clean-room manufactured **hanging drop plate seal** with an extra large 'window'. A new plate template provided with **mosquito®** allows the accurate and fast positioning of 3 low volume drops within each window of the new plate seal. This enables the simultaneous screening of different conditions within each well of a standard 96-well plate.

**Contact us for more information on anything featured here:**
Phone: +44 (0) 1763 262626 • Email: sales@ttplabtech.com
Fax: +44 (0) 1763 261964 **www.ttplabtech.com/mosquito**

Cover images courtesy of the Structural Biology Laboratory, University of York.

# The SGC: structural gen

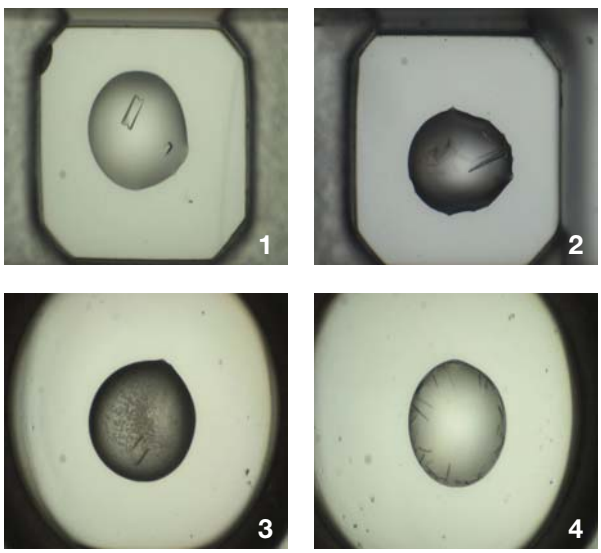
*Frank von Delft, Principle Investigator, Protein Crystallography Group, Structural Genomics Consortium, Oxford.*

**With only a few nanograms of protein necessary to form a crystal that can yield its full atomic structure, protein crystallography (PX) is by far the most sensitive and accurate technique available for the determination of protein structures and has proved particularly useful for drug discovery scientists.**

In recent years a series of spectacular technical advances have turned talk and funding to high-throughput crystallography and structural genomics. This in turn has lead to increased investment in technology development and in particular, has provided the impetus for the commercialisation of high-tech instrumentation - a tremendous benefit to the PX community as a whole. As a consequence, off-the-shelf technology has been the mainstay of PX infrastructure at the SGC-Oxford, where we have sought close collaboration with vendors to tailor instrumentation to our specific needs.

## The Structural Genomics Consortium

The SGC is a four-year, internationally-funded initiative operating from research laboratories at the University of Oxford, UK, the University of Toronto, Canada, and the Karolinska Institute in Stockholm. Its primary aim is to solve structures of medically relevant protein targets and place them in the public domain, along with experimental details and functional and binding data generated in the process. The SGC worldwide has set itself the goal to solve over 380 protein structures between July 2004 and June 2007, including proteins associated with cancer, neurological disorders and malaria.




1: MAP2K5-MAP3K3 PHOX DOMAIN COMPLEX 2: MAT1
3: JMJD2A 4: SLC9A3R2 DOMAIN 1

## Therapeutic targets: a family approach

Although genes can often be identified as underlying various diseases, it is the proteins they code for that are often the actual biological agents, leading to disease when they malfunction, *e.g.* deregulated key metabolic or signalling pathways. Through studying the basic biology – and structures – of all components of such a pathway, the range of therapeutic targets can often be broadened considerably. Furthermore, additional structural knowledge of other family members of a target should allow the rational design of drugs with greater specificity.

Here at Oxford, ~1000 targets were selected for study, of which we hoped to solve approximately 180 within our three year operational period. The targets cover about 40 structural families, most of which have members that are either involved in disease, or are relevant to other important biological systems. They fall into three broad functional areas: metabolic enzymes, phosphorylation-dependent signalling, and transmembrane receptor signalling.

The family approach has had practical benefits, because methodologies and reagents (*e.g.* ligand libraries) tend to be applicable across a family, thus increasing time- and cost-efficiency as well as success rate. Additionally, within families it is not only structure that tends to be conserved but general function too, providing us with a starting point for investigating the mechanism of action for less well characterised targets identified by the Human Genome Project (HGP).

## Experimental approach

As far as the crystallisation is concerned, rather than investing in the development of new methodologies, we have deliberately relied on existing, tried-and-tested experimental procedures, focusing instead on how to deploy them efficiently enough to support a high output. For instance, for our initial crystallisation screens, we have relied on commonly-used screening conditions, but using a reduced subset (2x96 conditions). This simplifies automation logistics, leaving more protein available for subsequent optimisation screens or additional co-crystallisation trials.

At the same time, we cannot sacrifice experimental flexibility. Targets routinely require more varied protocols than can be supported by standardisation and automation. The alternative is to process even more variations of sample (different constructs/various ligand co-crystallisations *etc.*), letting "statistical genius" take care of success. However, that leads to increased costs and lower efficiency, so our challenge is to find the balance between standardised many-sample protocols and experimental best practice.

# omics, the practical way

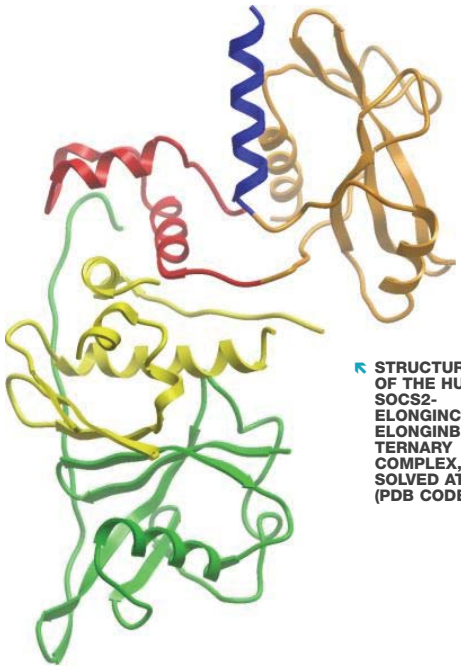
## Leveraging technology

As the SGC was only recently established, we have had the opportunity of using and optimising automation tools that were already on the market, developed on the back of the first wave of structural genomics initiatives. As a result, the majority of our crystallisation solutions are mixed by the MPII system (Perkin Elmer), interfaced by a set of home-grown scripts. This applies to both coarse and fine screens, resulting in improved reproducibility as well as savings. Nanodrop crystallisation is by now well established, and has enabled us to reduce manual workload and increase experimental throughput, and robotic pipetting also appears to help with repeatability of crystallisations. We use the TTP LabTech's mosquito® nanolitre pipettors, which have the advantage of ease-of-use and quick plate-to-plate turn-around. Plates are stored in and inspected automatically by the MinstrelIII plate incubators (RoboDesign), with a number of inspections over a period of two months to monitor crystal growth.

The primary use of our in-house X-ray diffractometer and the attached sample mounting robot is to screen crystals before they are shipped for synchrotron data collection. In-house sample changers are more typically employed for routine data collection, especially in pharmaceutical research. However, in our set-up its power lies in allowing meaningful ranking of even marginal crystals, thereby hugely increasing efficiency of beamtime usage at the synchrotron (for us, the Swiss Light Source).

The main guiding principle in how we've deployed our robotics has been to make them accessible but also effective, both experimentally and in throughput. It has required close collaboration with vendors. For example, with mosquito®, feedback from TTP LabTech engineers allowed us to optimise both volume of wasted protein and drop shape. A couple of technologies, in particular the mosquito®, have enabled us to leverage small quantities of proteins, and thereby lower the cost and effort of protein production. mosquito's ability to accurately pipette nanolitre volumes allows for much lower protein consumption for crystallisation, and its flexible experiment design is particularly powerful for optimisation screens.

Perhaps the best measure of the usefulness of a technology is internal acceptance, in which case we have been successful. Almost no crystallisations are set up by hand anymore, and almost all plates are submitted for automatic imaging. The technologies have thus provided clear benefits to our laboratories and to the every-day life of the people working in them.



STRUCTURE OF THE HUMAN SOCS2-ELONGINC-ELONGINB TERNARY COMPLEX, SOLVED AT 1.9Å (PDB CODE:2C9W)

## Moving forward

The SGC, as a whole, has generated 22% of all "unique" human structures in the public database so far in 2007, and has contributed over 340 structures since July 2004, including a number of protein-protein complexes. It has done so on budget and several months ahead of schedule. Despite having to maintain a high output of structures, our emphasis has been on the quality of results produced, which was helped by freeing up scientists' time by using high-tech systems for crystallisation, such as TTP LabTech's mosquito®. A next step now is to examine how our particular setup and methodologies are portable to different lab setups.

*This article was originally published in European Pharmaceutical Review, Issue 4, 2006.*

## Find out more about mosquito®

TTP LabTech is exhibiting at the 2007 Annual Meeting of the American Crystallographic Association (ACA), 21-27 July, Salt Lake City. Visit us to see **mosquito®** in action for yourself, and talk to our team to see how **mosquito®** could help automate your protein crystallography set-up.

For more information about **mosquito®** or any of TTP LabTech's products or services, please contact us on sales@ttplabtech.com or visit www.ttplabtech.com/mosquito. The first 20 enquiries marked "LabCrystal" drawn on August 20th will receive a TTP LabTech T-shirt.

# Improving protein crystallography efficiency at AstraZeneca

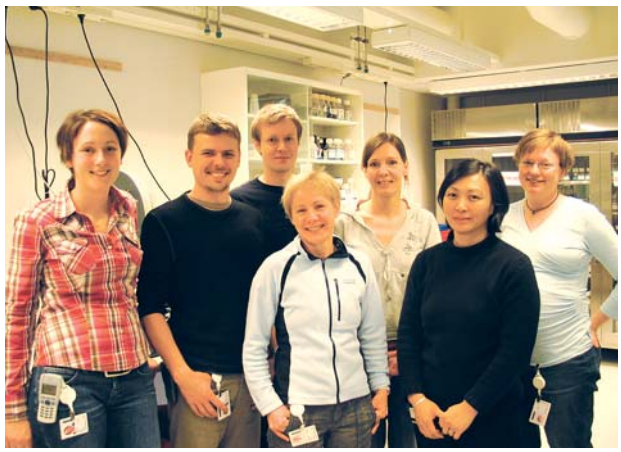
**Protein crystallography is now a major part of the drug discovery process for many pharmaceutical companies and the ability to automate methods has proved vital to the day-to-day success of the laboratories involved. Here, we talk to Lisa Wissler, MSc, a research scientist from the crystallisation team based at AstraZeneca (AZ), Mölndal, Sweden, about the group's recent progress and the steps they have taken to modify, automate and improve their crystallography set-up.**

Global Structural Chemistry (GSC) is a department within AZ which is divided across two sites, Mölndal, Sweden and Alderley Park, England. Each site consists of three sections - protein engineering, biomolecular NMR and protein structure, and it is the latter that encompasses the protein crystallisation and crystallography teams. Projects are allocated to the department from many different research areas (RAs) within AZ. As a part of the crystallisation team Lisa is responsible for a number of stages within the crystallisation process including: protein characterisation; screening for crystals; optimising hits; producing and testing crystals for diffraction; and sometimes collecting data prior to the handover to a crystallographer for structure determination.

## Protein crystallography evolution - automation is key

Crystallisation has always been referred to as the bottleneck in the gene-to-structure process and due to the nature of the science and the optimisation processes involved, has often been labelled a "trial and error" science. All of which has spurred on Lisa in her research. "It is funny, but this reputation has only served to motivate me further. Technology has advanced significantly and we are using this to our advantage. We want to decrease the timelines for crystallisation and increase our success rate."

Just five years ago all crystallisation-related work in the AZ Mölndal lab was carried out using manual methods. Today the picture is quite different and undoubtedly reflects the progress of many PX labs across the globe. The crystallisation lab is now fully automated and capable of handling the increasing amount of projects and more complex targets that are commonly bestowed upon them. The team have invested in equipment to improve each stage of the experimental process from screen design to crystal imaging and data capture. As part of this initiative the lab has invested in two of TTP LabTech's mosquito® nanolitre pipettors, one to operate at 20°C and one at 4°C.



↑ **FROM LEFT TO RIGHT: LISA WISSLER, FREDRIK MAURITZSON, CRISTIAN JOHANSSON, MARGARETA EK (TEAM LEADER), ANNA AAGAARD, HONGWEI GUO, LINDA ÖSTER**

"We use the mosquitos for both sitting and hanging drop techniques; the latter if a detergent is included in the protein buffer." Lisa said, "The mosquitos are fast, user-friendly and require very little maintenance which are real advantages. Many people in the lab work with the mosquitos and their ease-of-use has made the transition from manual to automated methods very smooth indeed."

"The crystallisation process itself has not changed drastically but the automation has radically improved the experimental process. mosquito® has allowed for a much faster and more accurate set-up of drops, and many experiments are more easily implemented e.g. seeding into a new 96-well screen, lowering the threshold greatly. The cost for setting up a 96-well plate with mosquito® is lower than setting up four 24-well plates by hand and the amount of protein used is also reduced considerably – even if the number of drops set up has significantly increased since the arrival of the mosquitos."

> "mosquito's ease of use has made the transition from manual to automated methods very smooth indeed"

According to Lisa all the new robotics help the team on a daily basis, providing people with more experimental freedom. There is not a strict crystallisation plan imposed for each project, but instead the team at Mölndal work to build tailored plans for each protein based on previous experimental trends and parameters. "Moving from a manual set-up to an automated lab has not only led to faster delivery of crystals but consequently, to the determination of more protein structures." Lisa said, "With an increased number of structures we can influence and aid the RA projects with more informed structure-based decisions and thereby hopefully contribute to accelerated drug discovery."

# Using hanging drop at UCLA

**Professor Jeff Abramson leads the structural biology team in the Physiology Department of the David Geffen School of Medicine, University of California, Los Angeles (UCLA). Known as a centre of excellence, the UCLA attracts many high-calibre scientists to its facilities. Researchers fall under 20 primary faculties, with key strengths in the areas of membrane biophysics, neurophysiology and cardiovascular research. Collaborations between research groups are common and Jeff works closely with other structure biology groups, to share not only expertise but also a number of valuable resources.**

With a rich scientific background, Jeff's particular expertise lies in membrane protein biochemistry and structure biology, specifically focusing on the structural determination of a number of membrane transport proteins. These include the $NA^{+}/Ca^{2+}$ exchanger (NCX) and the $Na^{+}$/glucose co-transporter family (SGLT). Both are extremely important, biologically-relevant targets that can be linked to a number of conditions, such as heart failure (NCX), and metabolic disorders (SGLT), amongst others. Thus, their structural identification could have a significant impact on the development of new therapeutics.

## Why membrane proteins?

Membrane proteins are critical to numerous biological functions and cellular activities. Consequently, they are highly relevant therapeutic targets and are currently the focus of approximately 50% of all marketed drugs. Although they represent 20-30% of all proteins in sequenced genomes, to date membrane proteins represent only about 0.6% of identified structures - so demand for structural information is high. However, it is widely known that these proteins can often present practical challenges. Membrane proteins reside in a phospholipid bilayer and as such are hydrophobic in nature. This makes them difficult to express, purify and crystallise. As a result Jeff's primary aim was to improve technologies both for the over-expression of specific membrane proteins and for the optimisation of their crystallisation conditions.

## Positive impact

Jeff also wanted to increase the overall throughput of his crystallisations and thus invested in some new instrumentation to expedite this process. Jeff predominantly employs the hanging drop technique for his crystallisations and today uses TTP LabTech's mosquito®, optimised for small to medium throughput. In one week the entire UCLA structure biology group set up 10 different proteins using 7-8 different commercial kits for soluble proteins and a selection of homemade kits optimised for membrane proteins. Jeff's usual set-ups, using mosquito®, consist of a 3-drop (per well of a 96 well flat-bottomed plate) hanging drop experiment, using protein ratios of 2:1, 1:1 and 1:2.


↑ **(LEFT TO RIGHT) STANDING: VINCENT CHAPTAL, JEFF ABRAMSON, RACHNA UJWAL, DANIEL (SEUNGHYUG) KWON, GABRIEL MERCARDO, AKIRA WATANABE. SITTING: SALEM FAHAM, MICHAEL HAHN, JENNIFER WARFEL**

mosquito® has allowed the dispensing of much smaller protein volumes and reagents for any given experiment and this has had significant cost benefits for Jeff's lab.

Furthermore, mosquito® has the ability to carry out precise additive screening into the hanging drops. This is of great benefit as optimisations of these conditions are without doubt, time-consuming, involving the individual application of both the additives and detergents to the crystallisation condition. With 192 additives and detergents this is no mean feat, especially if carried out by hand. However, using mosquito® for additive screens means this process can now be carried out in a fraction of the time with better reproducibility and accuracy than ever before. Jeff's lab have now increased their crystallisation rate 10 fold compared to old manual methods, testing 10 times the number of proteins compared to one year ago.

In summary, implementing instrumentation such as mosquito® has accelerated the crystallisation of highly relevant protein targets, including the SGLT family and the calcium-binding domains of the $NA^{+}/Ca^{2+}$ exchanger (NCX). Jeff commented, "mosquito® has had a major impact on our institution and it's success has spurred others to adopt this instrumentation. In fact, the entire UCLA crystallographic community now uses mosquito® including the tuberculosis consortium, to structurally identify three different TB proteins and another team, to optimise conditions of forced protein dimers. Additionally, several amyloid forming peptides have been successfully crystallised using a 3-drop experiment with mosquito®." Based on this success and the high quality service provided by TTP LabTech, another core crystallography group will soon be getting their own mosquito®.

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